

WHAT IS CLAIMED IS:

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1. A purified mammalian RapR7 protein.

2. The protein of claim 1 which comprises the amino acid sequence substantially as set forth in SEQ ID NO:4 or 7.

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3. A purified protein encoded by a nucleic acid capable of hybridizing to a DNA comprising a sequence consisting of the coding region of SEQ ID NO:2 or 5.

4. A purified derivative or analog of the protein of claim 1, which displays one or more functional activities of a mammalian RapR7 protein.

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5. The derivative or analog of claim 4 which is capable of binding to an antibody directed against a mammalian RapR7 protein.

6. A purified fragment of a mammalian RapR7 protein.

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7. The fragment of claim 6, wherein said fragment comprises a PHD domain of a mammalian RapR7 protein.

8. The fragment of claim 7, wherein said mammalian RapR7 protein is a human RapR7 protein, and wherein said fragment comprises amino acids 217-263 or 326-381 of said RapR7 protein.

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9. The fragment of claim 6, wherein said fragment comprises a coiled-coil domain of a mammalian RapR7 protein.

10. The fragment of claim 9, wherein said mammalian RapR7 protein is a human RapR7 protein, and wherein said fragment comprises amino acids 163-190 of said RapR7 protein.

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11. The fragment of claim 6, wherein said fragment comprises a second peroximal domain of a mammalian RapR7 protein.

12. The fragment of claim 11, wherein said mammalian RapR7 protein is a human RapR7 protein, and wherein said fragment comprises amino acids 514-522 of said RapR7 protein.

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13. A molecule comprising the fragment of any one of claims 6-12.
- 5 14. A protein comprising an amino acid sequence that has at least 60% identity to a domain of a mammalian RapR7 protein, in which the percentage identity is determined over an amino acid sequence of identical size to the domain.
- 10 15. A protein comprising an amino acid sequence that has at least 90% identity to a domain of a mammalian RapR7 protein, in which the percentage identity is determined over an amino acid sequence of identical size to the domain.
- 15 16. A polypeptide comprising a fragment of a mammalian RapR7 protein consisting of at least 6 amino acids fused via a covalent bond to an amino acid sequence of a second peptide, wherein said second peptide is not comprised in a mammalian RapR7 protein.
17. The polypeptide of claim 16, wherein the fragment of the mammalian RapR7 protein is a fragment capable of binding to an anti-RapR7 protein antibody.
- 20 18. The polypeptide of claim 17, wherein the fragment capable of binding to an anti-RapR7 protein antibody further lacks one or more domains of the RapR7 protein.
19. An antibody which is capable of binding to a mammalian RapR7 protein.
20. The antibody of claim 19 which is a monoclonal antibody.
- 25 21. A molecule comprising a fragment of the antibody of claim 19, wherein said fragment is capable of binding to a RapR7 protein.
22. An isolated nucleic acid comprising a nucleotide sequence encoding a mammalian RapR7 protein as set forth in SEQ ID NO:2 or 5.
- 30 23. The nucleic acid of claim 22 which is DNA.
24. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 22.
- 35 25. An isolated nucleic acid hybridizable to the nucleic acid of claim 22.
26. An isolated nucleic acid comprising a fragment of a mammalian RapR7 gene consisting of at least 8 nucleotides.

27. An isolated nucleic acid comprising a fragment of a mammalian RapR7 gene
5 comprising any one of exons 1-11 of a mammalian RapR7 gene.

28. An isolated nucleic acid comprising a fragment of a mammalian RapR7 gene
comprising an intron, or a fragment thereof, of a mammalian RapR7 gene.

29. An isolated nucleic acid comprising a nucleotide sequence encoding a fragment
10 of a mammalian RapR7 protein that displays one or more functional activities of the
mammalian RapR7 protein.

30. An isolated nucleic acid comprising a nucleotide sequence encoding any one of
the fragments of claims 6-12.

15 31. A recombinant cell containing the nucleic acid of claim 29.

32. A method of producing a mammalian RapR7 protein comprising:

(a) growing a recombinant cell containing the nucleic acid of
20 claim 29 such that the encoded fragment of said mammalian
RapR7 protein is expressed by the cell; and

(b) recovering said expressed fragment of said mammalian RapR7
protein.

25 33. The product of the process of claim 32.

34. A pharmaceutical composition comprising a therapeutically effective amount of
a mammalian RapR7 protein and a pharmaceutically acceptable carrier.

35. A pharmaceutical composition comprising a therapeutically effective amount of
30 an antibody capable of binding to a mammalian RapR7 protein and a pharmaceutically
acceptable carrier.

36. A method for generating a genetically modified cell having altered sensitivity to
rapamycin, said method comprising introducing into the genome of a cell of a selected cell
35 type of an organism a knockout DNA construct, said knockout DNA construct comprising (i)
a regulated promoter and (ii) a selection marker coding sequence under the control of said
regulated promoter, wherein said regulated promoter, when activated, initiates RNA
transcription to produce an RNA; wherein, when said regulated promoter is activated, said

genetically modified cell is rapamycin resistant if cells of said selected cell type is rapamycin
5 sensitive or is rapamycin sensitive if cells of said selected cell type is rapamycin resistant.

37. The method of claim 36, wherein said knockout DNA construct further
comprises a rapid cloning element comprising a replication origin sequence comprising
sequences for initiation of replication and segregation and a bacterial selection marker.

10 38. The method of claim 37, wherein said replication origin sequence is an Ori and
said bacterial selection marker is a chloramphenicol resistance gene.

39. The method of claim 36, wherein said method further comprising activating said
regulated promoter and identifying said genetically modified cell by a method comprising
15 identifying a change in rapamycin resistance in said genetically modified cell.

40. The method of claim 37, further comprising cloning a fragment of genomic
sequence by a method comprising: (a) obtaining a nucleotide sequence comprising said
rapid cloning element and said fragment of genomic sequence; (b) circularizing said
20 nucleotide sequence to generate a circular plasmid; and (c) transforming a suitable host cell
using said circular plasmid.

41. The method of claim 39, further comprising determining the sequence of said
fragment of genomic sequence by a method comprising sequencing said circular plasmid.

25 42. The method claim 40, further comprising determining the location of said
fragment of genomic sequence in said genome of said cell by a method comprising
comparing said sequences with the genomic sequence of said selected cell type.

43. The method of claim 40, wherein said method further comprising, prior to said
step of introducing said knockout DNA construct, introducing into the genome of cells of
30 said selected cell type a DNA construct encoding a transactivator, said DNA construct
comprising (i) a promoter and (ii) a nucleotide sequence encoding a transactivator, said
nucleotide sequence being under the control of said promoter, wherein said regulated
promoter is activated by said transactivator, and wherein said genetically modified cell is
generated by introducing said knockout DNA construct into a cell comprising said DNA
35 construct encoding said transactivator.

44. The method of claim 43, wherein said regulated promoter is a tetracycline
5 regulated promoter, and wherein said transactivator activates said regulated promoter in the
absence of tetracycline.

45. The method of claim 44, wherein said knockout DNA construct further
comprises a rapid cloning element comprising a replication origin sequence comprising
10 sequences for initiation of replication and segregation and a bacterial selection marker.

46. The method of claim 45, wherein said replication origin sequence is an Ori and
said bacterial selection marker is a chloramphenicol resistance gene.

47. The method of claim 46, wherein said method further comprising identifying
15 said genetically modified cell by a method comprising identifying a change in rapamycin
resistance in said genetically modified cell.

48. The method of claim 46, further comprising cloning a fragment of genomic
sequence by a method comprising: (a) obtaining a nucleotide sequence comprising said
20 rapid cloning element and said fragment of genomic sequence; (b) circularizing said
nucleotide sequence to generate a circular plasmid; and (c) transforming a suitable host cell
using said circular plasmid.

49. The method of claim 48, further comprising determining the sequence of said
fragment of genomic sequence by a method comprising sequencing said circular plasmid.
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50. The method of claim 49, further comprising determining the location of said
fragment of genomic sequence in said genome of said cell by a method comprising
comparing said sequences with the genomic sequence of said selected cell type.

51. The method of any one of claims 36-50, wherein said selected cell type is a
30 rapamycin sensitive cell type.

52. The method of claim 51, wherein said organism is a human.

53. The method of claim 51, wherein said organism is a mouse.

54. The method of claim 53, wherein said selected cell type is the murine
35 neuroblastoma N2a cell line.

55. The method of claim 51, wherein said knockout DNA construct is integrated at a
5 location in a RapR7 gene.

56. The method of claim 55, wherein said knockout DNA construct is integrated
before the coding region of said RapR7 gene such that the expression of said RapR7 gene is
activated or enhanced.

10 57. The method of any one of claims 36-50, wherein said selected cell type is a
rapamycin resistant cell type.

58. The method of claim 57, wherein said organism is a human.

15 59. The method of claim 57, wherein said organism is a mouse.

60. A method for treating a mammal having a cancer, said cancer being caused by
defective regulation of a RapR7 gene and/or defective activity of a protein encoded by said
RapR7 gene, said method comprising administering to said mammal a therapeutically
sufficient amount of an agent, said agent regulating the expression of said RapR7 gene
20 and/or activity of said protein encoded by said RapR7 gene.

61. The method of claim 60, wherein said cancer is caused by an increase of
expression of said RapR7 gene, and wherein said agent reduces the expression of said
RapR7 gene in cells of said cancer.

25 62. The method of claim 60, wherein said cancer is caused by a mutation in said
RapR7 gene, and wherein said agent causes the expression of a normal version of said
RapR7 gene in cells of said cancer.

63. A method for treating a mammal having a cancer, comprising administering to
30 said mammal a therapeutically sufficient amount of an agent, said agent regulating the
expression of a RapR7 gene and/or activity of a protein encoded by said RapR7 gene such
that rapamycin resistance is regulated, wherein said mammal is subject to a therapy
comprising administering to said mammal a therapeutically sufficient amount of rapamycin
or an analog or derivative of rapamycin.

35 64. A method for treating a mammal having a cancer, comprising administering to
said mammal i) a therapeutically sufficient amount of an agent, said agent regulating the
expression of a RapR7 gene and/or activity of a protein encoded by said RapR7 gene such

that rapamycin resistance is regulated, and ii) a therapeutically sufficient amount of
5 rapamycin or an analog or derivative of rapamycin.

65. The method of claim 63 or 64, wherein said agent reduces the expression of said
RapR7 gene in cells of said cancer.

66. The method of claim 63 or 64, wherein said agent causes the expression of a
10 normal version of said RapR7 gene in cells of said cancer.

67. The method of claim 63 or 64, wherein said agent comprises a RapR7 protein or
a therapeutically equivalent fragment thereof.

68. A method for diagnosing a cancer or a predisposition to said cancer in a
15 mammal, said cancer being a result of defective regulation of a RapR7 gene, said method
comprising determining an expression level of said RapR7 gene in cells of said mammal,
wherein said expression level below a predetermined threshold level indicates that said
mammal has or is predisposed of said cancer.

69. The method of claim 68, wherein said expression level of said RapR7 gene is
20 determined by a method comprising measuring the expression level of said RapR7 gene
using one or more polynucleotide probes, each of said one or more polynucleotide probes
comprising a nucleotide sequence in said RapR7 gene.

70. The method of claim 69, wherein said one or more polynucleotide probes
25 comprise at least one polynucleotide probe comprising a nucleotide sequence within one of
exons 1-11 of said RapR7 gene.

71. The method of claim 69, wherein said one or more polynucleotide probes
30 comprise at least one polynucleotide probe comprising a nucleotide sequence of said RapR7
gene which is not comprised in a nucleotide sequence that encodes a PHD domain, a coiled-
coil domain, a second peroximal domain, a nuclear localization domain or a low complexity
domain of the encoded RapR7 protein.

72. The method of claim 69, wherein said one or more polynucleotide probes
35 comprise at least one polynucleotide probe comprising a nucleotide sequence within an
intron of said RapR7 gene.

73. The method of claim 69, wherein said one or more polynucleotide probes
5 comprise at least one polynucleotide probe comprising a nucleotide sequence comprised in
the nucleotide sequence encoding a PHD domain, a coiled-coil domain, a second peroximal
domain, a nuclear localization domain or a low complexity domain in the encoded RapR7
protein.

10 74. The method of any one of claims 68-73, wherein said one or more
polynucleotide probes are polynucleotide probes on a microarray.

75. A method for diagnosing a cancer or a predisposition to said cancer in a
mammal, said cancer being a result of defective regulation of a RapR7 gene, said method
comprising determining a level of abundance of a protein encoded by said RapR7 gene in
15 cells of said mammal, wherein said level of abundance of said protein above a
predetermined threshold level indicates that said mammal has or is predisposed of said
cancer.

76. A method for diagnosing a cancer or a predisposition to said cancer in a
20 mammal, said cancer being a result of defective regulation of a RapR7 gene, said method
comprising determining a level of activity of a protein encoded by said RapR7 gene in cells
of said mammal, wherein said activity level above a predetermined threshold level indicates
that said mammal has or is predisposed of said cancer.

25 77. The method of claim 75 or 76, wherein said mammal is a human.

78. The method of claim 77, wherein said protein is a human RapR7 protein as
depicted in SEQ ID NO:6 or 7.

79. The method of claim 75 or 76, wherein said mammal is a mouse.

30 80. The method of claim 77, wherein said protein is murine RapR7 protein as
depicted in SEQ ID NO:3 or 4.

81. A method for evaluating rapamycin resistance in a cell, said method comprising
determining an expression level of a RapR7 gene in said cell, wherein said expression level
35 above a predetermined threshold level indicates that said cell is rapamycin resistant.

82. The method of claim 81, wherein said expression level of said RapR7 gene is
determined by a method comprising measuring the expression level of said RapR7 gene

using one or more polynucleotide probes, each of said one or more polynucleotide probes
5 comprising a nucleotide sequence in said RapR7 gene.

83. The method of claim 82, wherein said one or more polynucleotide probes
comprise at least one polynucleotide probe comprising a nucleotide sequence within one of
exons 1-11 of said RapR7 gene.

10 84. The method of claim 82, wherein said one or more polynucleotide probes
comprise at least one polynucleotide probe comprising a nucleotide sequence of said RapR7
gene which is not comprised in a nucleotide sequence that encodes a PHD domain, a coiled-
coil domain, a second peroximal domain, a nuclear localization domain or a low complexity
domain of the encoded RapR7 protein.

15 85. The method of claim 83, wherein said one or more polynucleotide probes
comprise at least one polynucleotide probe comprising a nucleotide sequence within an
intron of said RapR7 gene.

20 86. The method of any one of claims 81-85, wherein said one or more
polynucleotide probes are polynucleotide probes on a microarray.

87. The method of claim 86, wherein said one or more polynucleotide probes
comprise at least one polynucleotide probe comprising a nucleotide sequence comprised in
the nucleotide sequence encoding a PHD domain, a coiled-coil domain, a second peroximal
25 domain, a nuclear localization domain or a low complexity domain in a RapR7 protein.

88. A method for evaluating rapamycin resistance in a cell, said method comprising
determining a level of abundance of a protein encoded by a RapR7 gene in said cell,
wherein said level of abundance of said protein above a predetermined threshold level
30 indicates that said cell is rapamycin resistant.

89. A method for evaluating rapamycin resistance in a cell, said method comprising
determining a level of activity of a protein encoded by a RapR7 gene in said cell, wherein
said activity level above a predetermined threshold level indicates that said cell is
rapamycin resistant.

35 90. The method of claim 88 or 89, wherein said cell is a human cell.

91. The method of claim 90, wherein said protein is a human RapR7 protein as
5 depicted in SEQ ID NO:6 or 7.

92. The method of claims 88 or 89, wherein said cell is a murine cell.

93. The method of claim 92, wherein said protein is murine RapR7 protein as
depicted in SEQ ID NO:3 or 4.
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94. A method for regulating rapamycin resistance in a cell, comprising contacting
said cell with a sufficient amount of an agent such that rapamycin resistance is regulated,
said agent regulating the expression of a RapR7 gene and/or the activity of a protein
encoded by said RapR7 gene.

15 95. A method for regulating rapamycin resistance in a mammal, comprising
administering to said mammal a therapeutically sufficient amount of an agent such that
rapamycin resistance is regulated, said agent regulating the expression of a RapR7 gene
and/or the activity of a protein encoded by said RapR7 gene.

20 96. A method for regulating growth of a cell, comprising contacting said cell with i)
a sufficient amount of an agent such that rapamycin resistance is regulated, said agent
regulating the expression of a RapR7 gene and/or the activity of a protein encoded by said
RapR7 gene; and ii) a sufficient amount of rapamycin or an analog or derivative of
rapamycin.

25 97. The method of claims 94, 95 or 96, wherein said agent reduces the expression of
said RapR7 gene in said cell.

98. The method of claims 94, 95 or 96, wherein said agent causes the expression of
a normal version of said RapR7 gene in said cell.
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99. The method of claims 94, 95 or 96, wherein said agent comprises a RapR7
protein or a therapeutically equivalent fragment thereof.

100. A method of identifying an agent that is capable of regulating rapamycin
resistance, wherein said agent is capable of modulating the expression of a RapR7 gene
35 and/or the activity of a protein encoded by said RapR7 gene, said method comprising
comparing inhibitory effect of rapamycin on cells expressing said RapR7 gene in the
presence of said agent with inhibitory effect of rapamycin on cells expressing said RapR7

gene in the absence of said agent, wherein a difference in said inhibitory effect of
5 rapamycin identifies said agent as capable of regulating rapamycin resistance.

101. A method of identifying an agent that is capable of regulating rapamycin resistance, wherein said agent is capable of modulating the expression of a RapR7 gene and/or activity of a protein encoded by said RapR7 gene, said method comprising:

10 (a) contacting a first cell expressing said RapR7 gene with rapamycin in the presence of said agent and measuring a first growth inhibitory effect;

(b) contacting a second cell expressing said RapR7 gene with rapamycin in the absence of said agent and measuring a second growth inhibitory effect; and

15 (c) comparing said first and second inhibitory effects measured in said step (a) and (b),

wherein a difference between said first and second inhibitory effects identifies said agent as capable of regulating rapamycin resistance.

20 102. The method of claims 100 or 101, wherein said agent comprises a molecule which reduces expression of said RapR7 gene.

103. The method of claims 100 or 101, wherein said agent causes the expression of a normal version of said RapR7 gene in a cell.

25 104. A method of producing an antibody that binds specifically to a RapR7 protein, comprising raising said antibody against said RapR7 protein or a polypeptide comprising an fragment of said RapR7 protein.

30 105. The method of claim 104, wherein said RapR7 protein is a human RapR7 protein.

106. The method of claim 104, wherein said RapR7 protein is a murine RapR7 protein.

35 107. The method of claim 104, wherein said fragment of said RapR7 protein comprises a PHD domain, a coiled-coil domain, a second peroximal domain, a nuclear localization domain or a low complexity domain of said RapR7 protein.

108. The method of claim 107, wherein said fragment comprises amino acids 217-
5 263 or 326-381 of said RapR7 protein, or a fragment thereof.

109. An antibody that binds specifically to a RapR7 protein or a fragment of said RapR7 protein such that binding of said antibody to said RapR7 protein regulates rapamycin resistance.

110. The antibody of claim 109, wherein said RapR7 protein is a human RapR7 protein.

111. The antibody of claim 109, wherein said RapR7 protein is a murine RapR7 protein.

112. The antibody of claim 109, wherein said fragment of said RapR7 protein comprises a PHD domain, a coiled-coil domain, a second peroximal domain, a nuclear localization domain or a low complexity domain of said RapR7 protein.

113. The method of claim 109, wherein said fragment comprises amino acids 163-
20 190 or 514-522 of said RapR7 protein, or a fragment thereof.

114. An agent that regulates the expression of a RapR7 gene such that rapamycin resistance is regulated.

115. The agent of claim 114, wherein said agent comprises a molecule which
25 regulates expression of said RapR7 gene.

116. The agent of claim 115, wherein said molecule reduces expression of said RapR7 gene.

117. The agent of claim 114, wherein said agent blocks or reduces the binding of a
30 regulator to said RapR7 gene.

118. The agent of claim 117, wherein said regulator is an activator of said RapR7 gene.

119. The agent of claim 114, wherein said agent causes the expression of a normal
35 version of said RapR7 gene in a cell.

120. A cell comprising a knockout DNA construct at a RapR7 locus, said knockout
5 DNA construct comprising (i) a regulated promoter and (ii) a selection marker coding
sequence under the control of said regulated promoter, wherein said regulated promoter,
when activated, initiates RNA transcription of said RapR7 gene.

121. The cell of claim 120, further comprising a DNA construct encoding a
10 transactivator, said DNA construct comprising (i) a promoter and (ii) a nucleotide sequence
encoding said transactivator, said nucleotide sequence being under the control of said
promoter, wherein said transactivator activates said regulated promoter.

122. The cell of claim 120, wherein said knockout DNA construct further comprises
a rapid cloning element comprising a replication origin sequence comprising sequences for
15 initiation of replication and segregation and a bacterial selection marker.

123. The cell of claim 122, wherein said replication origin sequence is an Ori and
said bacterial selection marker is a chloramphenicol resistance gene.

124. The cell of claim 123, wherein said regulated promoter is a tetracycline
20 regulated promoter, and wherein said transactivator activates said regulated promoter in the
absence of tetracycline.

125. The cell of any one of claims 120-124, wherein said cell is a rapamycin
sensitive cell.

25 126. The cell of claim 125, wherein said cell is a human cell.

127. The cell of claim 125, wherein said cell is a murine cell.

128. The cell of claim 127, wherein said cell is a murine neuroblastoma N2a cell.

30 129. The cell of claim 127, wherein said integration site is in the intron between
exon 1 and exon 2 of said RapR7 locus.

130. The cell of any one of claims 120-124, wherein said cell is a rapamycin
resistant cell.

35 131. A microarray for diagnosing rapamycin resistance, said microarray comprising
one or more polynucleotide probes, wherein each said polynucleotide probe comprises a
nucleotide sequence in a RapR7 gene.

132. The microarray of claim 131, wherein said one or more polynucleotide probes
5 comprise at least one polynucleotide probe comprising a nucleotide sequence within one of
exons 1-11 of said RapR7 gene.

133. The microarray of claim 131, wherein said one or more polynucleotide probes
comprise at least one polynucleotide probe comprising a nucleotide sequence within an
10 intron of said RapR7 gene.

134. A kit for diagnosis of rapamycin resistance, comprising in one or more
containers one or more polynucleotide probes, wherein each said polynucleotide probe
comprises a nucleotide sequence in a RapR7 gene.

15 135. A kit for screening for agents which regulate rapamycin resistance and/or
tumorigenesis, comprising in one or more containers (i) the cell of claim 120; (ii)
tetracycline or a derivative or analog thereof; and (iii) rapamycin or a derivative or analog
thereof.

20 136. The protein of claim 1 which comprises the amino acid sequence substantially
as set forth in SEQ ID NO:3 or 6.

137. The polypeptide of claim 18, wherein said one or more domains of the RapR7
protein are selected from the group consisting of a PHD domain, a coiled-coil domain, a
second peroximal domain, a nuclear localization domain and a low complexity domain of
25 said RapR7 protein.

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